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FAP 1M4727

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date August 2, 2002

From Division of Petition Review (HFS-265)
Chemistry Review Team

Subject **FAP 9M4682** (MATS 2.0 and 2.1): National Fisheries Institute and Louisiana Dept. of Agriculture and Forestry (petition filed 5 October 1999 and amendment of 25 April 2001): Ionizing Radiation for the Pasteurization of Fresh or Frozen Molluscan Shellfish, *and*
FAP 1M4727 (MATS 2.0 and 2.1): National Fisheries Institute (Petition filed 11 January 2001 and amendment of 25 April 2001): Use of Approved Sources of Ionizing Radiation as a Physical Process to Reduce the Food Safety Risk in Consuming Crustaceans

To Division of Biotechnology and GRAS Notice Review (HFS-255)
Attention: Lane Highbarger, Ph.D.

I. Introduction

The National Fisheries Institute (NFI) and Louisiana Department of Agriculture have submitted two petitions to amend 21 CFR Part 179 - *Irradiation in the Production, Processing and Handling of Food*, to provide for the safe use of sources of ionizing radiation to irradiate refrigerated or frozen mollusks, and fresh, partially cooked, cooked, or processed crustaceans and crustacean food products to reduce the microbial load on and prolong the shelf life of these foods. The petitioners originally requested that the sources listed under §179.26(a) be permitted for the irradiation of mollusks at doses not to exceed 7.5 kGy (FAP 9M4682), and doses not less than 2 kGy for fresh crustaceans, 3 kGy for frozen crustaceans, or 0.7 kGy for lobster (FAP 1M4727). In a letter dated 25 April 2001, NFI amended its request for the maximum permitted dose for mollusks to 5.5 kGy. In a separate letter of 25 April 2001, NFI amended its petition for crustaceans by removing its proposed minimum doses and proposing a maximum permitted dose of 6 kGy.

Irradiation is currently permitted for pathogen reduction in a variety of foods under §179.26(b), notably red meat and poultry products (to 3 kGy for fresh / refrigerated poultry, to 4.5 kGy for fresh/refrigerated meat, and to 7 kGy for frozen meat), dried spices and seeds (to 30 kGy), and dried enzyme preparations (to 10 kGy). Fruits and vegetables may be irradiated at doses up to 1 kGy (§179.26 (b)(3)) to delay the onset of ripening, and food in general may be irradiated at doses up to 1 kGy, for disinfestation of arthropod pests.

This memorandum will comment on the chemistry aspects of this petition, including the proposed maximum permitted doses, relevant aspects of lipid chemistry and organoleptic quality,[1] and issues more specific to shellfish, such as the effects of irradiation on fatty acids and specific nutrients in shellfish. A variety of techniques for food preservation have been, and currently are in use. These methods, many of which have a long history of use in food preparation, also can chemically alter the foods to which they are applied.[2] The effects of irradiating food should, therefore, be compared with those of other food treatment processes, such as heat pasteurization and sterilization, acidity regulators, chemical preservatives, drying/dehydration, and freezing.

II. Sources of Ionizing Radiation

The sources of the ionizing radiation currently approved under §179.26(a) are radioisotopes (Cobalt-60 and Cesium-137 gamma ray sources); machine sources of electron-beams (up to 10 MeV); and x-ray generators (up to 5 MeV).

III. Technical Effect, Use, and Use Level

The petitioners seek to broaden the regulations to permit irradiation of certain shellfish products. NFI has requested the use of ionizing radiation to control Vibrio *spp.* and other food borne pathogens in fresh or frozen molluscan shellfish (FAP 9M4682) and fresh, frozen, or processed crustaceans (FAP 1M4727). In particular, the petitioners assert that ionizing radiation will inactivate all *Vibrio* present, and reduce levels of coexisting *Salmonella* and *Listeria* species, which may be present in shellfish, thereby reducing the potential for food poisoning from this food source. Issues pertaining to technical effect and efficacy in the current submissions will be addressed by CFSAN microbiologists.

The molluscan shellfishes covered by FAP 9M4862 include fresh or frozen clams, oysters, scallops, and mussels, either shucked or still in the shell. The crustacean shellfishes covered by FAP 1M4727 include shrimp, prawns, lobster, and crawfish/crayfish. Crustaceans covered by this petition include those that are raw, frozen, cooked (with or without shell), or prepared with other minor ingredients, such as breading or spices (ready-to-cook or ready-to-eat). The chemistry aspects of irradiating non-meat items (such as batter or breading), including food additives, have been addressed elsewhere, and will not be discussed further in this memorandum.[3]

Much work has been carried out in an effort to determine optimum doses and conditions for the irradiation of seafood.[4,5,6] A monograph by the International Consultative Group on Food Irradiation (ICGFI)[7] summarizes a great deal of the research on optimum and maximum doses used in seafood irradiation studies. Maximum recommended doses for most shellfish fall in the range of up to 2 to 2.5 kGy (Armstrong et al 1994),[8] but must be determined for each species, and depend on factors such as storage and processing conditions, and the amount of oil in the fish. For example, flounder fillets (about 1.2% lipid content[9]) can tolerate doses of 4.5 kGy, but sardines - much oiler fish (lipid content about 12%[10]) - are better treated at 0.23 kGy.[11] For mollusks, whether the product is in the shell or not can significantly affect the optimum dose. Unshucked products can tolerate doses up to 8 kGy, but shucked mollusks tolerate about 2-4 kGy. Most crustaceans can tolerate doses up to about 2 kGy,[12,13] although, as noted in the petition, lobster appears to be more radiation-sensitive, and cooked lobster meat is best irradiated at doses between 0.75 kGy and 2 kGy.

IV. Dosimetry

To ensure that foods receive an adequate dose to accomplish the intended technical effect and to comply with regulatory limits, methods are needed to monitor the dose of irradiation delivered to the target. These methods typically use dosimeters to show that food has been irradiated within the desired range (dosimetric methods). Relevant ISO/ASTM Standard Guides and Practices are available for users of dosimetry systems.[14]

V. Effects of Ionizing Radiation on Foods

A. Radiation Chemistry - General Considerations for Food Irradiation

Foods consist of three principal components (other than water): carbohydrates, proteins, and lipids. Radiation will interact directly with these components, which may induce chemical reactions ("direct" effects). At the petitioned doses, however, radiation will also cleave (photolyze) water to produce various free radicals (·H, ·OH), which subsequently react with the other food components. These "secondary" reactions are responsible for most of the physical and chemical changes seen in irradiated foods.[15]

The effects of radiation processing on the characteristics of the treated foods are a direct result of the chemical reactions induced by the absorbed radiation. Research has established that the types and amounts of products generated by radiation-induced reactions (hereinafter referred to as "radiolysis products") depend on the chemical constituents of the food and on the conditions of irradiation (i.e., temperature, water content of the food, presence or absence of oxygen, dose, and dose rate). Scientists have compiled a considerable amount of data regarding the effects of ionizing radiation on different foods under various conditions of irradiation.[16] The amounts of radiolysis products generated in a particular food have been shown to be directly proportional to the agency has recognized that "from the basic principles of radiation chemistry, the irradiation of the meat, within broad dose limits and irradiation temperatures, will affect only the relative concentration of the radiolysis products ... the types [of radiolysis products] ... have already been addressed in the review of FAP 4M4428."[25] As shellfish is a member of the "flesh foods" category, then, these conclusions remain applicable to the proteins in shellfish and fish.

2. *Lipids*

Some of the major changes that occur during processing, distribution, and final preparation of food are due to oxidation of lipids.[26] Oxidation of lipids initiates other changes in the food system that may affect nutritional quality, wholesomeness, safety, color, flavor, and texture.[27] Reviews of food irradiation have concluded that the auto-oxidative processes induced by irradiation in fat are essentially the same as those that occur normally without irradiation, but at an accelerated rate.[28]

The chemistry of lipids in general, and those in meats specifically, was considered by the agency in the final rules authorizing the use of radiation to process meat and poultry, and in the reviews of a petition to irradiate shell eggs (FAP 8M4584). Primary oxidation products of fats and fatty acids include monohydro-peroxides. Secondary products include carbonyl compounds that carry flavor and odor, small fatty acids (aerobic rancidity), and hydrocarbons.[29] A variety of end-products in irradiated lipids has been identified, including fatty acids, esters, aldehydes, ketones, and various hydrocarbons.[30] Fats and lipids are susceptible to oxidation when not irradiated, and the same types of oxidation processes also occur in non-irradiated foods.[31]

C. Chemistry of Polyunsaturated Fatty Acids (PUFAs)

One major difference between fish and shellfish and other flesh foods is that fish and shellfish generally contain more polyunsaturated fatty acids (PUFAs). PUFAs are a subclass of lipids that have more than one double bond in the hydrocarbon chain. PUFAs generally are considered to be more readily oxidized than saturated fatty acids[32] due to the presence of the reactive double bonds. Therefore, PUFAs may be expected to react preferentially compared to other lipid components, leading both to decreased PUFA levels in irradiated products and increased formation of radiolysis products. The formation of off-aromas and off-colors, and other organoleptic changes are generally accepted to be due to the action of oxygen on unsaturated fatty acids.[33]

Studies of irradiation of certain fish, however, have led to the conclusion that irradiation products derived primarily from saturated and mono-unsaturated fatty acids; few products can be attributed to PUFAs.[34] This is consistent with work on other flesh foods, in which irradiation of meats at doses up to 10 kGy did not appear to result in a decrease in double bonds nor result in oxidative damage to unsaturated lipids.[35]

The question of possible destruction of PUFAs has been studied by a number of pasteurization or sterilization and deep freezing, can alter the chemical constituents of foods, including nutrients. In some cases, these treatments can reduce the content of sensitive (labile) vitamins such as thiamine, even more than irradiation.[51] Therefore, incorporating irradiation into shellfish processing will not necessarily result in a net decrease of nutrients in those foods or in the diet.

E. Mutual Protection

The concept of "mutual protection"[52] basically states that ingredients in mixtures undergo fewer changes than ingredients that are irradiated neat or in aqueous solutions. For example, a sugar solution is an inadequate model for the extent of changes that take place in irradiated fruit, as it predicts neither the reaction products nor their concentrations well. Foods usually are complex mixtures and the relative reactivity of the many different components toward free radicals is not adequately modeled by such simplistic models. Pure lipids, for example, are not good candidates for irradiation because they are affected negatively (oxidation, rancidity). It is clear, however, that meats, which are relatively high lipid-content foods, can survive irradiation without undue effects on the lipid fraction. Meat irradiation studies have shown a protective effect of proteins against the oxidative effects on lipids.[53]

F. Self-limiting Use Levels - Organoleptic Changes

Applied doses for irradiation are self-limiting due to the changes in organoleptic properties (color, taste, odor) that may occur in foods. Physical and organoleptic changes - changes in odor, texture, and appearance - result from the breakdown or oxidation of lipids and proteins. Additionally, the breakdown even of minor constituents can lead to changes that could make a product unacceptable to consumers.

According to Diehl,[54] salmon, for example, is unlikely to be irradiated because the carotenoid pigments that give fillets their characteristic pink color are bleached by irradiation. The high lipid content of salmon flesh (> 10% for raw farmed Atlantic salmon[55]) makes this species an unlikely candidate for irradiation, since at the doses needed for pathogen reduction (e.g., D_{10} doses for various organisms on shrimp range from 0.1 kGy for *Vibrio* to 3 kGy for *C. botulinum* spores)[56], the fish is rendered hedonistically or organoleptically unacceptable.[57] Shrimp, too, may be bleached by irradiation, resulting in an altered appearance.[58] Compounds that can be detected by smell or taste are also formed during irradiation, such as the carbonyl compounds that may result from the oxidation of alcohols and ethers. These compounds, many of which are naturally-occurring flavoring substances, impart perceptible taste and odor at very low levels, again resulting in a product that could be unacceptable to consumers.[59] Although such effects would be reduced by irradiating the product while frozen, there may be times when it is undesirable to freeze the product, e.g., when the vendor wishes to market fresh, unfrozen fillets. Irradiation of frozen products is discussed in more detail below.

As noted above, the lipids commonly found in fish are expected to be oxidatively labile, and the maximum suggested doses for irradiation for many marine foods are about 2-2.5 kGy (Armstrong et al, 1994, page 352).[8] For example, linoleic acid can produce decadienal (Patton *et al.* 1959, cited in Lacroix et al) and unsaturated C:18 fatty acids can produce alkanals, alk-2-enals, and alk-2,4-dienals (Ramaswamy and Richards, 1982)[60]. They also cited a study that showed that the amount of volatile hydrocarbons increases linearly with the absorbed dose (Lesgards et al, 1993, in Lacroix et al); so it is reasonable to expect an increasing odor at higher radiation doses. In another study increased lipid oxidation (rancidity), browning and poorer organoleptic qualities of dried fish irradiated at doses up to 5 kGy was observed.[61] Thus, we conclude that the doses used for shellfish will, in most cases be self-limiting because of changes in taste, aroma, and appearance that may occur at doses above about 2-3 kGy.

G. Water Content and Mobility

Although lipids principally interact with incident radiation and undergo chemical reactions as a result ("direct effects"), many chemical changes in foods take place because of secondary reactions with products of water radiolysis. As a consequence, water content and radical mobility will affect the degree to which food components will react. All other factors being equal, foods with high water content will undergo more chemical changes than foods that have low water content (e.g., dried). Similarly, foods which are irradiated at high temperatures will undergo more chemical changes than those at low temperatures (e.g., frozen). Thus, dehydration and freezing can have a protective effect, making foods less susceptible to radiation-induced changes in organoleptic properties than their hydrated or room-temperature counterparts.[62]

Foods with high water content undergo much more radiolytic damage, due to the reactions associated with water radiolysis products, than do foods with relatively low water content. The reduced "reactivity" of dried and frozen foods has, in the past, influenced the regulatory limitations on the maximum dose for different foods. The different limitations reflect the higher sensitivity of products that contain water and are not frozen compared to those that are frozen or dried due to temperature (freezing decreases ion mobility) and decreases in reactivity due to less water content.

H. Oxygen

The presence or absence of oxygen can also affect the degree to which irradiation may induce chemical changes in foods. Typically, fewer changes accompany irradiation of foods under a modified atmosphere (e.g., low oxygen).[63] Therefore, it is likely that certain marine foods would normally be irradiated under these conditions. For example, in their study of antioxidant effects on the degree of oxidation of lipids during food irradiation, Lacroix *et al.* (1997) showed that significantly fewer products were detected when linoleic acid (18:2) is irradiated under nitrogen than under air.[64] In a separate study, irradiation of cod and plaice in air at 200 krad (2 kGy) led to organoleptically unacceptable products, and the authors concluded that the fish would likely better tolerate this dose under vacuum or under and inert atmosphere.[65]

In a study of irradiated mackerel, the authors found that oxidative degradation of mackerel during storage was about the same for fresh fish and processed fish. They interpreted this as an indication that irradiation had not resulted in any significant differences in terms of oxidizing any components of the fish compared to unirradiated fish.[66]

I. Other Considerations

While irradiation can increase the shelf life of processed products (as measured by chemical and sensory metrics), irradiation alone cannot serve as a food-preservation technique. Rather, foods that have been irradiated must be subject to the same post-irradiation treatment and handling as their unirradiated counterparts.[67] Thus, irradiation is not a substitute for good manufacturing practices, but rather, serves an additional step for processing and handling food in accordance with GMP or as part of a HACCP program.

J. Proposed Dose Limits

In two amendments dated 25 April 2001, NFI proposed a maximum permitted absorbed dose for molluscan shellfish of 5.5 kGy and a maximum permitted absorbed dose of 6 kGy for crustacean shellfish. These doses are below the maximum permitted absorbed dose for frozen red meat (7.5 kGy). Although the proposed regulation does not state that crustaceans that might be irradiated to this dose should be frozen, based on the data described in earlier sections it is likely that only frozen shellfish could absorb this dose and retain their "fresh" organoleptic qualities. Thus, non-frozen shellfish are expected to be irradiated at lower doses than the proposed maxima. We have no questions regarding the proposed maximum dose limits for shellfish.

VI. Exposure Considerations

After evaluating the radiation-induced chemistries of water, protein, and lipids (the major constituents of flesh foods), FDA has concluded that the products formed are the same as or similar to those that are found in non-irradiated foods, for example, after cooking.[68,69] These products are typically oxidation products of the constituents of those foods. Even at elevated temperatures, when reaction rates are higher, the amounts of radiolytic products formed are much smaller than by other heat processing methods (e.g., cooking). We further note that the radiolysis products in shellfish will be essentially the same as those in red meat and poultry, since the composition of all of these foods is roughly the same. Since shellfish typically make a smaller contribution to the average daily diet, the exposure to radiolysis products from shellfish will be commensurately smaller than the exposure to those compounds from foods for which irradiation is already regulated. Therefore, cooking or other heat processing methods will remain the principle means for introducing such substances into the diet.

VII. Proposed Regulation

The petitioners offered regulatory language for inclusion in the CFR in the petitions, and proposed maximum doses and removed the requested minimum doses in the amendments of 25 April 2001. Based on these proposals, we suggest that the following language be used to amend 21 CFR 179.26:

Use	Limitations
* * *	* * *
9. For control of pathogens in uncooked fresh or frozen mollusks, in shell or shucked (including clams, oysters, scallops, and mussels)	Absorbed dose not to exceed 5.5 kGy.
10. For control of pathogens in raw, fresh, frozen, cooked, partially cooked, shell-on or shelled, dried, and/or processed crustaceans (including crab, crayfish / crawfish, lobster, shrimp, and prawn).	Absorbed dose not to exceed 6.0 kGy.

VIII. Summary

In summary, we have considered the effects of expanding the permitted uses of ionizing radiation to include control of pathogens in molluscan and crustacean shellfish. Based on the fact that (a) irradiation of other flesh foods, e.g., red meat and poultry, has been determined to be safe; (b) the products generated by irradiating flesh foods are essentially the same as those created during cooking; (c) the amounts of radiolysis products created are much smaller than the amounts of the same compounds formed during cooking or other heat-treatments; and (d) the maximum requested doses are no greater than the dose permitted for frozen red meat; we conclude that the irradiation of shellfish up to the maximum requested doses raises no chemistry questions. Consumers of irradiated shellfish would not be exposed to new or significantly more radiolysis products, nor would the nutritional adequacy of the diet be compromised.

Food processors who use ionizing radiation to treat these foods should ensure that they are irradiated under appropriate conditions that will minimize the effects of the radiation on the food. For example, oxygen should be excluded when appropriate, and shellfish should be irradiated at the lowest practicable temperature. Conditions should be optimized so that foods absorb the minimum dose necessary to accomplish intended technical effect.

Daniel E. Folmer, Ph.D.

for

Elke Jensen, Ph.D.

(formerly of Division of Product Manufacture and Use, Chemistry and Exposure Assessment Team)

cc: HFS-226 HFS-245 (Morehouse/Diachenko) HFS-248 **FAP 9M4697**

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References

[1] See also the discussion of these points in the memorandum from E. Jensen, HFS-246, to L. Highbarger, HFS-206, of 7/25/00 (FAP 9M4697).

[2] See for example, JF Diehl and ES Josephson "Assessment of Wholesomeness of Irradiated Foods " Acta Alimentaria 23(2) (1994) 195-214. Page 202 lists vitamins affected by various methods of food processing e.g. heating, freezing, and irradiation.

[3] Memorandum from E. Jensen (HFS-246) to L. Highbarger (HFS-206) of 25 July 2000 (9M4697).

[4] International Consultative Group on Food Irradiation (ICGFI) "Monograph on Irradiation of Fish, Shellfish, and Frog Legs" (IAEA, Vienna, October, 1998) and references therein.

[5] (a) Shea K.M.; Balk S.J.; Gitterman B.A.; Miller M.D.; Shannon M.W.; Weil W.B.; Galson S.K.; Linet M.; Miller R.W.; Rogan W.; Coven B.; Bearer C.F.; Etzel R.A.; Goldman L.; Halbrook B.; Kaferstein F.; Keener K.; Thayer D.W.; Hall L.A. "Technical Report: Irradiation of Food," Pediatrics 106(6) (2000) Pages 1505-1510. (b) J. Farkas "Irradiation as a Method for Decontaminating Food: A Review," International Journal of Food Microbiology, Volume 44, Issue 3, 10 November 1998, Pages 189-204. (c) Lester M. Crawford and Eric H. Ruff "A Review of the Safety of Cold Pasteurization Through Irradiation," Food Control, 7(2) April 1996, Pages 87-97. (d) Andrews L.S., Ahmedna M, Grodner R.M., Liuzzo J.A., Murano P.S., Murano E.A., Rao R.M., Shane S, Wilson P.W. "Food Preservation Using Ionizing Radiation." Rev. Environ. Contam. Toxicol. 1998;154:1-53.

[6] J. Farkas "Irradiation as a Method for Decontaminating Food: A Review," Int. J. Food Microbiology, **44**(3) 10, November 1998, Pages 189-204.

[7] ICGFI monograph (ref. 4), section 10, "Seafood Irradiation Research" pp. 33-48.

[8] S.G. Armstrong, S.G. Wylie, and D.N. Leach, "Effects of Preservation by Gamma-Irradiation on the Nutritional Quality of Australian Fish," Food Chemistry **50** (1994) 351-357.

[9] U.S. Department of Agriculture, Agricultural Research Service. 1999. USDA Nutrient Database for Standard Reference, Release 13. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp

[10] *Ibid.* (USDA nutrient database)

[11] Mackerel irradiated at 1.5 kGy, for example, exhibited a decreased rate of spoilage and improved textural properties than control samples. S.V. Ghadi, M.D. Alur, V. Venugopal, S.N. Doke, S.K. Ghosh, N.F. Lewis, G.B. Nadkarni, Conference Proceedings: "Food Preservation by Irradiation" vol. I, (IAEA, Vienna, 1978) pp. 310, 313.

[12] See also, Y.P. Chen, L.S. Andrew, R.M. Grodner, "Sensory and Microbial Quality of Irradiated Crab Meat Products" J. Food Sci. **61** (1996) 1239, see page 1241.

[13] For shrimp irradiated at 100 krad (1 kGy): Houwing et al (IAEA conference proceeding).

[14] Published ISO/ASTM dosimetry methods include: ISO/ASTM 51204, Standard Practice for Dosimetry in Gamma Irradiation Facilities for Food Processing, ISO/ASTM

51261, Standard Guide for Selection and Calibration of Dosimetry Systems for Radiation Processing, ISO/ASTM 51431, Standard Practice for Dosimetry in Electron and Bremsstrahlung Irradiation Facilities for Food Processing, ISO/ASTM 51707 Standard Guide for Estimating Uncertainties in Dosimetry for Radiation Processing.

[15] J. F. Diehl, Safety of Irradiated Foods, 2nd edition, (Marcel Dekker, Inc., New York, 1995), page 71, pp. 242-247.

[16] See for example (a) "Recommendations for Evaluating the Safety of Irradiated Foods" (July 1980) Bureau of Foods Irradiated Food Committee Report (BFIFC Report). (b) Wholesomeness of Irradiated Food, World Health Organization, Geneva, 1981. (c) Safety and Nutritional Adequacy of Irradiated Food, World Health Organization, Geneva, 1994. (d) J.F. Diehl, "Safety of Irradiated Foods" 2nd Edition, Marcel Dekker, Inc., New York, NY, 1995.

[17] 62 F.R. 64107, 3 December 1997.

[18] (a) Memorandum from Chemistry Review Branch, FDA, to Biotechnology Policy Branch, (CFSAN/OPA) FDA, dated January 12, 1995. (b) Memorandum from Regulatory Food Chemistry Branch to Indirect Additives Branch, dated January 5, 1987. (c) Memorandum from Division of Product Manufacture and Use to Regulatory Policy Branch, dated February 23, 2000.

[19] Memorandum from Director, Office of Plant and Dairy Foods and Beverages, FDA to Biotechnology Policy Branch, (CFSAN/OPA) FDA, dated March 11, 1996.

[20] See for example, S. G. Armstrong, S. G. Wylie, and D.N. Leach, "Effects of Preservation by Gamma-irradiation on the Nutritional Quality of Australian Fish," Food Chemistry 50 (1994) 351-357.

[21] See for example, Health Effects of Dietary Fatty Acids, G.J. Nelson, ed., (AOCS, Champaign, IL, 1991); Fish Oils in Nutrition ME Stansby, ed., (Van Nostrand Reinhold, NY, 1990); Crawford M., Galli C., Visioli F., Renaud S., Simopoulos A.P., Spector A.A. "Role of Plant-derived Omega-3 Fatty Acids in Human Nutrition" Annals of Nutrition and Metabolism 44(5-6) (2000) Pages 263-265; Grundy S.M., "The optimal ratio of fat-to-carbohydrate in the diet " Annual Review of Nutrition Volume 19 (1999), Pages 325-341; Holman R.T. "The Slow Discovery of the Importance of 3 Essential Fatty Acids in Human Health" Journal of Nutrition 128(2 SUPPL.) (1998) Pages 427S-434S.

[22] 62 F.R. 64107, 3 December 1997.

[23] Memorandum from K.M. Morehouse (HFS-245) to L. Highbarger (HFS-206) of 10 Aug. 2001 (FAP 9M4697).

[24] Diehl, page 71.

[25] KM Morehouse (HFS-245) to R. Alrefai (HFS-206) 23 Feb. 2000 9M4695 and footnotes 4-8 therein.

[26] L.S.. Sant'Ana and J Mancini-Filho "Influence of the Addition of Antioxidants in Vivo on the Fatty Acid Composition of Fish Fillets" Food Chemistry 68 (2000) 175-178.

[27] F. Shahidi and P.D. Wanasudarda, "Phenolic Antioxidants" Crit. Rev. Food Sci. Nutr. 32 (1992) 67-103 (cited in Sant'ana paper, page 175).

[28] ICGFI, cited in Sant'ana, page 175.

[29] Food Chemistry, H.-D. Belitz, W. Grosch, eds. (Springer-Verlag Publ., Berlin, 1987), page 156.

[30] "Evaluation of Food Irradiation by Ionizing Radiation" vol. I (E.S. Josephson and M.S. Peterson Ed.), CRC press.

[31] See for example, W. W. Nawar, "Reactions Mechanisms in the Radiolysis of Fats: A Review" J. Agric. Food Chem. 26 (1978) 21-25; reference 9, pp. 72-80.

[32] "Nutritional Aspects of Food Irradiation: An Overview," E.S. Josephson, M.H. Thomas, W.K. Calhoun, J. Food Proc. and Preserv. 2 (1978) 299 - 303.

[33] I. Tukenmezy, M.S. Ersen, A.T. Bakiogu, A. Bicer, V. Parnok, Radiat. Phys Chem. 50(4) (1997) 407-414.

[34] Armstrong *et al.*

[35] J.W. Hampson, J.B. Fox, L. Lakritz, D.W. Thayer, "Effect of Low Dose Gamma Radiation on Lipids in Five Different Meats" Meat Science 42 (1996) 271-276.

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[37] Morehouse, KM; Ku, Y., "Gas Chromatographic and Electron Spin Resonance Investigations of Gamma-Irradiated Shrimp. J. Agric. Food Chem., 40(10), 1963-1971, 1992

[38] Morehouse, K.M., "Identification of Irradiated Seafood." in Detection Methods for Irradiated Foods: Current Status. McMurray, C.H.; Stewart, E.M.; Gray, R. and Pearce, J., eds., The Royal Society of Chemistry, Cambridge, UK, pp. 249-258, 1996.

[39] The notation n:m refers to the number of carbons (n) and the number of double bonds (m), where"ω" designates the position of the double bond closest to the terminal end of the hydrocarbon chain.

[40] S. B. Han, J. H. Lee, K. H. Lee, "Non-enzymic Browning Reactions in Dried Anchovy When Stored at Different Water Activities" Bull. Korean Fish. Soc., 6, 37, 1973.

[41] ICGFI, page 60.

[42] Thayer, D.W., "Food Irradiation: Benefits and Concerns." J. Food Quality, 13, 147-169, 1990.

[43] ICGFI, page 57.

[44] Even though vitamin E is radiation-sensitive, it will not be addressed in this review because fish and shellfish contribute less than 3% of the RDA for this nutrient. Memorandum from P. Hansen (HFS-006) to L. Highbarger (HFS-206), FAP 9M4697, in preparation.

[45] B. Underdal, J. Nordal, G. Linde, B. Eggen, "Effects of Ionizing Radiation on Nutritional Values of Mackerel" Lebensm.- u. Technol. 9 (1976) 72.

[46] "Nutritional Aspects of Food Irradiation: An Overview," E.S. Josephson, M.H. Thomas, W.K. Calhoun, J. Food Proc. and Preserv. 2 (1978), page 307.

[47] K. Lee, L. Hau, "Effect of γ-irradiation and Post-irradiation Cooking on Thiamine, Riboflavin and Niacin Contents of Grass Prawns (Penaeus monodon)" (1979) Food Chem. 55 (379-382).

[48] Technical Assessment Systems (TAS) International Diet System (Arlington, VA, 1997 v. 3.51). This is the average intake for eaters-only, based on the total US population.

[49] Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, B6, Folate, B12, Pantothenic Acid, Biotin, and Choline (National Academy of Sciences Press, Washington, DC, 1998).

[50] "Fatty Acid Composition of Fish" M.E. Stansby, H. Schlenk, E.H. Gruger in Fish Oils in Nutrition, M.E. Stansby, ed. (Van Nostrand Reinhold, NY, 1990), pp. 31-32.

[51] See for example, J.F. Diehl and E.S. Josephson "Assessment of Wholesomeness of Irradiated Foods " Acta Alimentaria 23(2) (1994). On page 202 the authors list vitamins affected by various methods of food processing e.g. heating, freezing, and irradiation; see also Josephson, Thomas, and Calhoun, page 307. (Ref. 1)

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[53] Diehl, page 78.

[54] Diehl

[55] USDA nutrient database.

[56] See for example, 1M4727, Section B, page 16 of 52, in which the petitioners list typical doses needed to achieve 10-fold reduction of various organisms. Higher doses, then would be needed to achieve higher log-reductions.

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[68] Radiolysis of water, breaking of peptide bonds, and radiation-induced cross-linking of polypeptide chains. See for example, 62 FR 64101, December 3, 1997 (red meat), 65 FR 45280, July 21, 2000 (shell eggs), 62 FR 64101, December 3, 1997 (poultry), and references therein.

[69] Memorandum from K. M. Morehouse, HFS-245, to W. Trotter, HFS-206, 14 May 1999 (FAP 8M4584).